CLAIMS

1. An isolated DNA molecule comprising at least a sequence A flanked by at least site specific recombinase targeting sequences (SSRTS) L1, and at least a sequence B flanked by at least site specific recombinase targeting sequences (SSRTS) L2, said SSRTS L1 and SSRTS L2 being unable to recombine with one another, and wherein:

(i) sequences L1 are in an opposite orientation, and

(ii) sequences L2 are in an opposite orientation, and

(iii) the order of SSRTS sequences in said DNA molecule is 5'-L1-L2-L1-L2-3'.

2. The DNA molecule according to claim 1, wherein the order of sequences in said DNA molecule is : 5'-L1-sequence A-L2-sequence B-L1-L2-3'.

3. The DNA molecule according to claim 1, wherein the order of sequences in said DNA molecule is : 5'-L1-L2-sequence A-sequence B-L1-L2-3'.

4. The DNA molecule according to claim 1, wherein the order of sequences in said DNA molecule is : 5'-L1-L2-sequence A-L1-sequence B-L2-3'.

5. The DNA molecule according to claims 1 to 4, wherein sequences A and B are in an opposite direction.

6. The DNA molecule according to claims 1 to 5, wherein the recombinase specific of said SSRTS L1 and the recombinase specific of said SSRTS L2 are the same.

7. The DNA molecule according to claims 1 to 5, wherein the recombinase specific of said SSRTS L1 and the recombinase specific of said SSRTS L2 are different.

8. The DNA molecule according to claims 1 to 7, wherein the recombinase specific of said SSRTS is selected from the group of site-specific recombinases composed of the Cre recombinase of bacteriophage P1, the FLP recombinase of Saccharomyces cerevisiae, the R recombinase of Zygosaccharomyces rouxii pSR1, the A recombinase of Kluyveromyces drosophilarium pKD1, the A recombinase of Kluyveromyces waltii pKW1, the integrase λ Int, the recombinase of the GIN recombination system of the Mu phage, of the bacterial β recombinase or a variant thereof.

9. The DNA molecule according to claim 8, wherein the recombinase is the Cre recombinase of bacteriophage P1 or its natural or synthetic variants.

10. The DNA molecule according to claim 9, characterized in that said SSRTS L1 and/or L2 specific for said Cre recombinase are chosen from the group composed of the sequences Lox P1, Lox 66, Lox 71, Lox

511, Lox 512, Lox 514 and mutated sequences of Lox P1 site harboring at least one point mutation in the spacer sequence.

11. The DNA molecule according to claim 10, wherein either SSRTS L1 comprises the Lox P1 nucleotide sequence (SEQ ID N° 1) and SSRTS L2 comprises the Lox 511 nucleotide sequence (SEQ ID N° 2) or SSRTS L1 comprises the Lox 511 sequence and SSRTS L2 comprises Lox P1 sequence.

12. The DNA molecule according to claim 8, wherein the recombinase is the FLP recombinase of Saccharomyces cerevisiae, or its natural or synthetic variants.

13. The DNA molecule according to claim 12, characterized in that said SSRTS L1 and/or L2 specific for said FLP recombinase are chosen from the group composed of the sequences FRT-S and FRT-$F3^{0.88}$.

14. The DNA molecule according to claims 1 to 13, wherein said DNA molecule is further flanked by at least site specific recombinase targeting sequences (SSRTS).

15. The DNA molecule according to claims 1 to 14, wherein said sequences A and B are selected in the group consisting of non transcribed sequence,

transcribed but not translated sequence, transcribed and translated sequence.

16. The DNA molecule according to claim 15, wherein at least the sequences A and/or B are transcribed and translated sequences coding for at least one protein selected in the group consisting of polypeptide, protein and protein fragments.

17. The DNA molecule according to claim 16, wherein said protein is selected in the group consisting of reporter protein, selection marker and protein of interest.

18. The DNA molecule according to claims 16 and 17, wherein sequences A and/or B are coding for at least one exon, or a fragment thereof.

19. The DNA molecule according to claim 18, wherein said exon differs from the wild type exon of a protein of interest by one or more point mutations.

20. The DNA molecule according to claims 16 and 17, wherein said protein is encoded by a cDNA sequence, and wherein an IRES sequence is inserted 5′, or 3′, or 5′ and 3′ to said cDNA sequence.

21. The DNA molecule according to claims 17 to 20, wherein said reporter protein is selected in the group consisting of autofluorescent proteins and enzymes detectable by a histochemical process.

22. The DNA molecule according to claim 21, wherein said autofluorescent protein is selected in the group consisting of the green fluorescent protein (GFP), the enhanced green fluorescent protein (EGFP), the red fluorescent protein (RFP), the blue fluorescent protein (BFP), the yellow fluorescence protein (YFP) and variant of these proteins.

23. The DNA molecule according to claim 21, wherein said enzyme, detectable by a histochemical process, is selected in the group consisting of β-galactosidase, β-glucoronidase, alcaline phosphatase, luciferase, alcohol deshydrogenase, chloramphenicol-acetyl transferase.

24. Vector comprising the isolated DNA molecule of claims 1 to 23.

25. Use of an isolated DNA molecule according to claims 1 to 23 or a vector according to claim 24 as a transgene.

26. Isolated transgenic host cell transformed by an isolated DNA molecule according to claims 1 to 23 or a vector according to claim 24.

27. Isolated transgenic host cell according to claim 26 wherein sequences of homology are present at both extremities of said DNA molecule.

28. Isolated transgenic host cell according to claim 27 wherein said isolated DNA molecule or said vector is integrated by homologous recombination in at least one targeted locus of the genome of said cell.

29. Isolated transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is integrated in sites of the genome chosen among polyA sites and gene promoters.

30. Isolated transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is randomly integrated in at least one locus of the genome of said cell.

31. Isolated transgenic host cell according to claim 26 wherein said isolated DNA molecule or said vector is maintained in said cell in an episomal form.

32. Transgenic organism, excepted humans, comprising at least one cell according to claims 26 to 31.

33. Method for the stable inversion of a DNA sequence comprising the steps of :

(i) contacting a DNA molecule according to claims 1 to 23, or a DNA vector according to claim 24 with at least one recombinase specific of said SSRTS L1 and one recombinase specific specific of said SSRTS L2 ;

(ii) inversion of said sequences A and B or sequence A or sequence B by recombination catalyzed by said recombinase at either SSRTS L1 or L2 sequences; and

(iii) excision by recombination catalyzed by said recombinase of a DNA fragment comprised in between the SSRTS L1 or L2 sequences that are now present in direct orientation following the inversion of step (ii), and that are able to recombine with one another.

34. Method according to claim 33 wherein said DNA fragment excised in step (iii) comprises the sequence A.

35. Method for obtaining a transgenic cell of which at least one allele of a DNA sequence of interest is invalidated by a process of conditional deletion and the genome of which comprises a gene selected among reporter gene, marker gene and gene encoding a protein of interest, inserted at the place of the DNA fragment deleted by said process of conditional deletion, said method comprises the steps of :

(i) Preparation of a DNA molecule according to claims 1 to 23 wherein sequence A or sequence B is coding at least for part of the DNA fragment of interest to be invalidated and sequence B or sequence A is coding at least for a reporter gene;

(ii) Obtention of a transgenic cell genetically modified by the targeted insertion by homologous

recombination at the place of said DNA sequence of interest, of a DNA molecule prepared at step (i) ;

(iii) Contacting said DNA molecule with at least one recombinase specific of SSRTS L1 and one recombinase specific of SSRTS L2 ;

(iv) Inversion of sequences A and B or sequence A or sequence B by recombination catalyzed by said recombinase at either SSRTS L1 or SSRTS L2 sequences ; and

(v) Excision of a DNA sequence by recombination catalyzed by said recombinase at SSRTS L2 or SSRTS L1 respectively, these SSRTS L2 or SSRTS L1 sequences being now present in direct orientation following to the inversion of step (iii), and being to recombine with one another.

36. Method of claim 35, wherein the order of sequences in said DNA molecule is 5'-L1-sequence A-L2-sequence B-L1-L2-3' and wherein a sequence of homology with the DNA sequence of interest are present at both extremities of said DNA molecule and wherein, the DNA fragment excised in step (v) comprises sequence A.

37. Method of claim 35, wherein the order of sequences in said DNA molecule is 5'-L1-L2- sequence A-sequence B-L1-L2-3' and wherein a sequence of homology with the DNA sequence of interest are present at both extremities of said DNA molecule.

38. Method of claim 35 wherein the order of sequences in said DNA molecule is 5'-L1-L2-sequence A-L1-sequence B-L2-3' and wherein a sequence of homologis with the DNA sequence of interest are present at both extremities of said DNA molecule.

39. Method to perform site-specific recombination mediated cassette exchange (RMCE), said method comprising the steps of :

(i) Preparation of a first DNA molecule comprising a first DNA sequence of interest flanked by incompatible SSRTS L1 and L2 in an opposite direction, obtainable by the method of claims 33 to 38 ;

(ii) Preparation of a second DNA molecule comprising a second DNA sequence of interest flanked by the same incompatible SSRTS L1 and L2 as in step (i) in an opposite direction, by *in vitro* DNA cloning;

(iii) contacting said first and said second DNA molecule with at least one recombinase specific of said SSRTS L1 and one recombinase specific specific of said SSRTS L2 ;

(iv) Exchange by recombination catalysed by said recombinase of said first and said second DNA sequence of interest comprised in between the SSRTS L1 and L2.

40. Method according to claim 39 wherein said second DNA molecule of step (ii) is obtainable by the method of claims 33 to 38.

41. Method according to claims 33 to 40 wherein the steps are made in a cell free system.

42. Method according to claims 33 to 40 wherein the steps are made in the cell of claims 26 to 31.

43. Method according to claim 42, further comprising the step of introducting into the cell a gene encoding the corresponding site-specific recombinase.

44. Method according to claim 43, wherein the gene encoding said site-specific recombinase is contained in an expression vector.

45. Method according to claim 43, wherein the gene encoding said site-specific recombinase is stably inserted into the genome of said cell.

46. Method according to claims 33 to 45, wherein either SSRTS L1 comprises the Lox P1 sequence and SSRTS L2 comprises the Lox 511 sequence, or SSRTS L1 comprises the Lox 511 sequence and SSRTS L2 comprises Lox P1 sequence, and wherein the corresponding site-specific recombinase is Cre or its material or synthetic variants.

47. Use of a DNA molecule according to claims 1 to 23, or a vector according to claim 24, or a cell

according to claims 26 to 31 to perform site-specific stable inversion of a DNA sequence.

48. Use of a DNA molecule according to claims 1 to 23, or a vector according to claim 24, or a cell according to claims 26 to 31 to perform site-specific recombination mediated cassette exchange (RCME).

49. Living organism, except humans, that comprises at least one transgenic cell obtainable by the method of claims 33 to 46.

50. Living organism of claim 49, wherein said organism is selected in the group consisting of bacteria, yeast, Caenorhabditis elegans, Drosophila melanogaster, zebrafish, mice, rat, rabbit, hamster, Guinea pig, cow, pig, goat, sheep, horse, primate.

51. Living organism of claim 50, wherein said organism is a mouse.

52. Living organism of claim 50, wherein the organism is a yeast.